Journal of Food Protection, Vol. 64, No. 11, 2001, Pages 1705–1710

Distribution of *Campylobacter* spp. in Selected U.S. Poultry Production and Processing Operations

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MS 01-83: Received 1 March 2001/Accepted 27 May 2001

ABSTRACT

A study was conducted of 32 broiler flocks on eight different farms, belonging to four major U.S. producers. The farms were studied over 1 complete calendar year. Overall, 28 (87.5%) of the flocks became *Campylobacter* positive, and only four (12.5%) remained negative throughout the 6- to 8-week rearing period. In the majority of flocks, sampled every 2 weeks throughout production, *Campylobacter*-positive fecal and cecal samples were not detected until 4 to 8 weeks of age. In only six of the flocks were environmental samples found to be positive before shedding of *Campylobacter* was detected in the birds. Even in some of the *Campylobacter*-negative flocks, contamination of the rearing environment was positive for *Campylobacter* but did not result in the birds subsequently excreting the organism. These findings are discussed in relation to U.S. husbandry practices and present uncertainty about sources of *Campylobacter* infection for poultry flocks. Birds were often transported to the processing plant in coops that were already contaminated with *Campylobacter,* and the organisms were sometimes found in samples of scald water and chill water. After chilling, the proportions of *Campylobacter*-positive carcasses from different producers ranged from 21.0 to 40.9%, which is lower than in other studies, and possible reasons are considered.

Campylobacter enteritis continues to be a significant public health problem throughout the world. In the United States alone, it is estimated that more than 2.4 million cases have been occurring annually, of which 80% are considered to be foodborne *(12).* Since broiler chickens are frequently asymptomatic intestinal carriers of *Campylobacter jejuni/coli* and the organisms are common contaminants of processed broiler carcasses, it is to be expected that poultry meat will be a vehicle of human campylobacteriosis. Consumer exposure to *Campylobacter* can occur if the meat is not handled hygienically, is not cooked properly prior to consumption, or both *(4).* Therefore, there is considerable interest in reducing *Campylobacter* infection in poultry production and diminishing levels of contamination on processed carcasses.

At present, efforts to develop more appropriate control measures are hampered by inadequate knowledge of the sources and modes of transmission of *Campylobacter* to poultry flocks. Various possibilities have been considered, including vertical transmission from parent to progeny via the egg *(5, 15)* and exposure of birds to contaminated water *(16),* a previously contaminated rearing environment *(10, 14, 23),* and other potential vectors, including rodents, insects, domestic pets, food animals, and farm personnel, as reviewed by Stern *(18).* In the processing plant, carcass contamination may be reduced by appropriate hygiene control measures *(11),* but there are numerous opportunities for cross-contamination of carcasses, and it is unlikely that *Campylobacter* will be eliminated by any means currently available.

There have been no national surveillance studies on *Campylobacter* in poultry, and little information is available in this respect for North America. The purpose of the present study was to gather such data for poultry operations that are representative of modern commercial conditions in specific areas of the United States and to identify prominent sources of flock infection.

MATERIALS AND METHODS

Flock location and husbandry conditions. The participating companies were located respectively in Alabama, Arkansas, California, and Georgia and were randomly coded A through D. Each producer provided a rearing house on two different farms, one with a history of high broiler-growth performance and the other associated with low performance. At each of the sites, separate broiler flocks were studied during the spring, summer, fall, and winter of 1998—a total of eight flocks per company.

Each broiler flock was comprised of ca. 20,000 birds, involving an "all-in, all-out" stocking policy with a rearing period of 6 or 8 weeks. Drinking water for the birds was obtained from a chlorinated mains supply, a nonchlorinated well, or both. In most cases, the litter on which the birds were kept was fully removed annually, when cleaning and disinfection of the houses were carried out. At other times, the top layer of used litter was removed ("decaking") and replaced with a layer of fresh litter ("top dressing"). By contrast, the houses of producer C were emptied and fully cleaned and disinfected between flocks or for every alternate flock. To increase ventilation during hot weather, the houses were partially opened to the outside, and, where required, water sprays were used to provide evaporative cooling.

All producers operated a rodent control program; however,

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little use was made of disinfectant foot baths, apart from producer C, who also made available clean, dedicated footwear. Most of the farms were close to other livestock, which sometimes had access to the site, while dogs, cats, or both were present on all farms, except for producer C.

Collection, transport, and examination of samples. All samples from both farms and processing plants were collected on each occasion within 1 to 2 h and transferred in insulated boxes containing ice packs for overnight transport to the laboratory. Where it was possible to collect and deliver samples on the same day, the samples were held under refrigeration overnight to ensure uniformity of sampling among farms. The microbiological tests were set up within 2 h.

Farm sampling and microbiological examination. On each farm and sampling occasion, samples were taken aseptically (i) just prior to placement of chicks, and (ii) at 2-week intervals thereafter, until the birds were sent for slaughter. The following samples were taken and examined, as appropriate, by the methods described below.

Delivery tray liners. Each of 25 paper liners from the chick delivery trays was placed in a separate 50- by 35-cm plastic bag, together with 500 ml of Difco buffered peptone water (BPW; Becton Dickinson, Sparks, Md.). After mixing the contents by hand, 10 ml of the resultant suspension was added to 90 ml of *Campylobacter* enrichment broth (CEB; Acumedia Manufacturers Inc., Baltimore, Md.) plus *Campylobacter*-selective supplement (Bolton's, Medox Diagnostics, Ogdensburg, N.Y.). The medium was incubated at 37°C for 4 h, followed by 42°C for 44 h. At 24 and 48 h, cultures were streaked on plates of Campy-Cefex agar *(20),* which was incubated at 42°C for 24 to 36 h in a microaerobic atmosphere containing 5% O_2 plus 10% CO_2 plus 85% N_2. Presumptive colonies of *Campylobacter* spp. were examined microscopically for typical motility and typical cell morphology and were confirmed with a latex agglutination test, Latex-CAMPY (Integrated Diagnostics Inc., Baltimore, Md.).

Unless stated otherwise, the same enrichment and plating procedures were used for the samples described below.

Chicken fecal samples. Twenty-five freshly excreted samples, each of approximately 5 g, were collected in whirl-pak bags and weighed. After adding 3 ml/g of BPW to each sample, the material was mixed by hand and plated (0.1 ml) on Campy-Cefex agar. The sample was also diluted 10^{-3} to 10^{-5} and plated on Campy-Cefex agar. All plates were incubated at 42°C for 34 to 36 h in a microaerobic atmosphere.

Cecal droppings. For each of five individual droppings, which were less abundant than feces, two cotton-tipped swabs were inserted into the center of the material, taking care to avoid external contamination. The swabs were transferred to 5 ml of BPW in a plastic bag, and the contents were mixed by hand. Then, 1 ml of the suspension was added to 90 ml of CEB plus supplement. In addition, 0.1 ml of the suspension was plated directly onto Campy-Cefex agar.

Water-line swabs. Six swab samples were taken from the interior of the house, within the water-supply pipes. Using cotton-tipped applicators, each swab was used to sample an area of ca. 10 cm^2 inside the pipe. After sampling, the swab was placed in a plastic bag with 5 ml of BPW and treated as for cecal droppings.

Drinker swabs. Six separate drinkers were sampled with four or five swabs in each case. Sampling covered the outside of each nipple drinker and the entire internal surface of its associated cup. The samples were treated as described for water-line swabs.

Litter. Each 6- by 10-g sample of dry litter was added to 90 ml of CEB plus supplement. Three samples were taken per side to ensure representative sampling of the house.

Feed hopper and feed. Two each of 10-g samples of feed were taken from (i) the feed hopper and (ii) individual feeders and were added directly to 90 ml of CEB plus supplement.

Drag swabs and swabs from walls and fans. Three drag swabs were taken with sterile gauze pads, each of which was used to sample a large part of the entire floor area of the house. In addition, two gauze-pad swabs each were taken from the walls of the house (ca. 100 cm^2) and the ventilation fans (ca. 100 cm^2). Each swab was placed in a separate plastic bag containing 10 ml of BPW, and the contents were mixed by hand. A 1-ml portion of the resultant suspension was transferred to 90 ml of CEB plus supplement.

Mouse samples. Mice were obtained either from traps placed just inside the house or from those found dead as a result of consuming mouse bait. Any live animal was first killed, placed in a plastic bag, and shaken in 100 ml of BPW for 1 min. A 10-ml portion of the rinse fluid was transferred to 90 ml of CEB plus supplement. Then, the carcass was rinsed in 70% alcohol and aseptically dissected to remove the intestinal tract, which was homogenized with a stomacher in 10 ml of BPW before adding 1 ml of the suspension to 90 ml of CEB plus supplement. In the case of dead animals, the whole carcass was homogenized in 100 ml of BPW, and 10 ml was added to 90 ml of CEB plus supplement.

Wild-bird feces. Where bird droppings outside the house were observed, each sample (1 to 5 g) was added to 10 ml of BPW in a plastic bag, and the contents were mixed by hand. From the suspension, 1 ml was added to 90 ml of CEB plus supplement.

Feces of other farm animals. Whenever livestock or pets were present, 10 g of feces was collected and added to 90 ml of CEB plus supplement. Also, the material was swab sampled and plated on Campy-Cefex agar.

Insects other than flies. Samples of live and dead insects were obtained either from inside the house or immediately outside. Those from the inside were mainly darkling beetles. Each sample was crushed in 10 ml of BPW, and 1 ml of the suspension was added to 90 ml of CEB plus supplement.

Flies. Usually, two samples were obtained from fly papers suspended in the house. Each sample was treated as described above for other insects.

Soil. A 10-g sample of surface soil was collected near the entrance to the house and was added to 90 ml of CEB plus supplement.

Standing water. Where water was present in the vicinity of the house, 10 ml was collected and added to 90 ml of CEB plus supplement.

Boots. Up to three swab samples were collected with sterile gauze pads from the boots of farm staff, when and where possible, or from those sampling on the farm. The swabs were treated as described for drag swabs and swabs from walls and fans.

Conditions of processing. All the processing plants were modern, highly mechanized operations, and each processed up to

TABLE 1. *Appearance of flock infection and proportions of* Campylobacter*-positive fecal and cecal samples on initial detection and just prior to slaughter of the flock*

Flock code[a]	Initially positive (week no.)	Positive samples (%)[b]			
		On initial detection		Preslaughter at week ( )	
		Fecal	Cecal	Fecal	Cecal
AHW	6	12.0	20.0	12.0 (6)	20.0 (6)
AHS	6	56.0	0	56.0 (6)	0 (6)
AHSu	4	28.0	100	28.0 (6)	60.0 (6)
AHF	6	40.0	60.0	40.0 (6)	60.0 (6)
ALW	4	88.0	60.0	40.0 (6)	20.0 (6)
ALS	6	100	80.0	100 (6)	80.0 (6)
ALSu	4	0	60.0	0 (6)	20.0 (6)
ALF	4	8.3	4.0	68.0 (6)	60.0 (6)
BHW	NF	NA	NA	0 (6)	0 (6)
BHS	8	88.0	80.0	88.0 (8)	80.0 (8)
BHSu	8	100	40.0	100 (8)	40.0 (8)
BHF	6	68.0	80.0	100 (8)	100 (8)
BLW	2	0	25.0	96.0 (6)	100 (6)
BLS	4	64.0	ND	100 (8)	80.0 (8)
BLSu	4	40.0	0	92.0 (8)	60.0 (8)
BLF	4	24.0	20.0	96.0 (8)	80.0 (8)
CHW	NF	NA	NA	0 (6)	0 (6)
CHS	6	88.0	100	88.0 (6)	100 (6)
CHSu	6	92.0	ND	92.0 (6)	ND (6)
CHF	6	8.0	100	8.0 (6)	100 (6)
CLW	NF	NA	NA	0 (6)	0 (6)
CLS	6	96.0	80.0	96.0 (6)	80.0 (6)
CLSu	6	96.0	80.0	96.0 (6)	80.0 (6)
CLF	6	8.0	100	8.0 (6)	100 (6)
DHW	6	100	100	100 (6)	100 (6)
DHS	6	60.0	100	60.0 (6)	100 (6)
DHSu	6	100	100	100 (6)	100 (6)
DHF	2	56.0	0	100 (6)	100 (6)
DLW	6	100	80.0	100 (6)	80.0 (6)
DLS	6	16.0	100	16.0 (6)	100 (6)
DLSu	6	40.0	80.0	40.0 (6)	80.0 (6)
DLF	NF	NA	NA	0 (6)	0 (6)

[a] Initial letter denotes producer codes A through D; second letter indicates flock on high (H) or low (L) performance site; final letters correspond to winter (W), spring (S), summer (Su), and fall (F). NA, not applicable; ND, no data; NF, not found.

[b] Percentages calculated from samples positive out of 25 for feces and 5 for cecal droppings.

7,000 carcasses/h. After scalding, plucking, and evisceration, the carcasses were spray washed and chilled in counterflow, water-immersion chillers. The input water to the chiller contained up to 50 mg/liter of free available chlorine.

Processing plant samples: transport coops before and after bird delivery. On each occasion, 10 gauze swab samples were taken and treated as described above for drag swabs and swabs taken from walls and floors. Each sample suspension was also diluted and plated onto Campy-Cefex agar, as described for chicken fecal samples.

Process water. Five samples were collected from (i) the scald tank, and (ii) the immersion chilling system before and after processing of the flock in question. In each case, a 10-ml amount of the water was added to 90 ml of CEB plus supplement.

TABLE 2. *Environmental samples found to be* Campylobacter *positive prior to the appearance of flock infection*

Flock code[a]	Week(s) before infection detected	Positive samples (%) in week(s) preceding infection
AHF	4	Drag swabs (100)
ALS	4	Mouse rinse (100), insects from house (25.0)[b]
ALF	0	Wild-bird feces (50.0)
DLW	4	Domestic animal feces (100)
DLS	2	Domestic animal feces (100)
DLSu	4	Drag swabs (100)

[a] Key as for Table 1.

[b] Animals caught externally.

Carcasses. Samples were taken after the passage of ca. 1,000 carcasses from the test flock. Each of 25 carcasses taken out of the final tank on completion of chilling was collected in a plastic bag and rinse sampled, as described by Cox et al. *(6)*. A portion of the rinse fluid (10 ml) was added to 90 ml of CEB plus supplement. Two further 0.1-ml amounts were plated on Campy-Cefex agar as before.

RESULTS

Data for all 32 flocks studied are summarized in Table 1. Flock codes have been abbreviated as follows: the initial letter denotes producer codes A through D; the second letter indicates flock on high (H) or low (L) performance site; and final letters correspond to winter (W), spring (S), summer (Su), and fall (F). The table shows that 28 (87.6%) were *Campylobacter* positive, and only four of the flocks were negative throughout the rearing period of 6 to 8 weeks. In 92.8% of the positive flocks, however, the appearance of infection was a relatively late event, being detected at 4 to 6 weeks of age or later for the one producer using an 8-week rearing cycle. For most of the flocks, there was evidence that the infection spreads among the birds with time, since the proportion of positive fecal and cecal samples increased up to the point of slaughter, sometimes reaching 100% in both cases. Exceptions were flocks AHSu, ALW, and ALSu, where the prevalence remained the same or diminished. There were also some marked discrepancies between fecal and cecal samples at particular sampling times, although one was not consistently a more sensitive measure of flock infection than the other.

Table 2 gives details of flocks in which environmental samples were found to be *Campylobacter* positive before flock infection was detected. Such samples were found in only six of the positive flocks (21.4%), and in only three of these did positive environmental samples come from inside the house, viz. drag swabs (AHF and DLSu) and insects (ALS). For the majority of infected flocks, there was no evidence of environmental contamination in the house itself or its immediate surroundings from which the infection could have arisen. By contrast, environmental contamination was readily apparent once the birds began shedding *Campylobacter* (data not shown). A further anomaly is that positive environmental samples were obtained from the in-

TABLE 3. *Environmental samples found to be* Campylobacter *positive for flocks that remained free from campylobacters*

Flock code[a]	Positive samples (%) during rearing period
BHW	Drag swabs (66.6), fan swabs (50.0)
CHW	Mouse intestines (50.0), wild-bird feces (50.0), standing water (100)[b]
CLW	Wild-bird feces (50.0)

[a] Key as for Table 1.
[b] Animals caught externally.

ternal environment of one of the flocks (BHW) that remained *Campylobacter* free (Table 3). With the two other negative flocks for which environmental samples were positive (CHW and CLW), the samples came from sources outside the houses and included mice, wild birds, and standing water, which may or may not have provided a source for colonization of the poultry within.

When data from all the flocks studied were combined and analyzed for any seasonal effect, it was found that the prevalence of positive fecal samples was greatest in the summer months (Fig. 1). However, a different situation was observed for cecal samples, with more of a spring predominance. The data were not subjected to statistical analysis because the study was only for 1 year. For flocks reared in houses with a history of high or low growth performance, comparisons of fecal and cecal material were made at different sampling times (Fig. 2). Despite a slightly higher prevalence of positive samples in houses associated with low growth performance, there was no marked effect of growth performance history on the frequency of *Campylobacter* spp. infection among flocks.

All flocks were followed through the processing plant, and it is evident in Table 4 that transport coops arriving on the farms from the processing plant were sometimes contaminated with *Campylobacter* spp. before being used to transport the flocks under study. Since most of the flocks in this investigation were *Campylobacter* positive, the coops were further contaminated during transportation of the birds and therefore were clearly a potential source of cross-contamination for other flocks. In the processing plant, both scald water and chill water were sometimes contaminated with *Campylobacter* spp. before the processing of one of the test flocks. This may have been due partly to prior processing of another flock, which was known to have occurred on three occasions, in spite of attempts to ensure that each test flock was processed first in the day. After chilling of carcasses in chlorinated water (up to 50 ppm chlorine), *Campylobacter*-positive carcass rinse samples from each producer ranged from 21.0 to 40.9%. For the flocks that had tested negative on the farm, only one (CHW) became contaminated during processing and yielded 12% positive rinse samples.

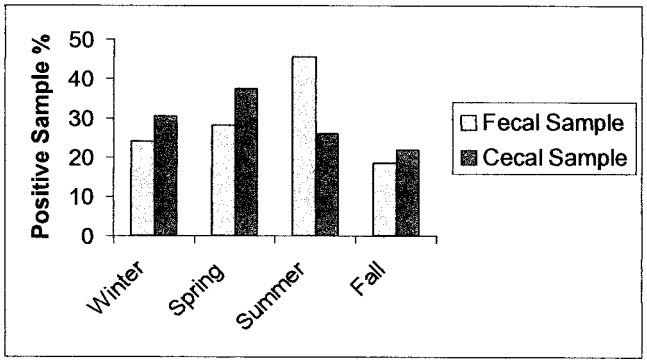

FIGURE 1. *Seasonal variation in the proportion of* Campylobacter-*positive fecal and cecal samples (all flocks and rearing weeks combined).*

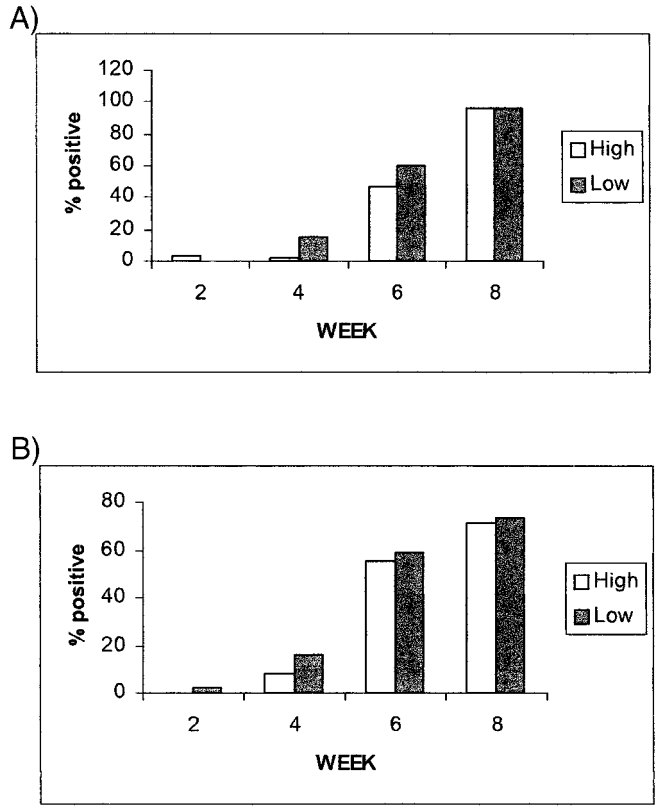

FIGURE 2. *Comparison of high and low bird-performance farms in relation to proportions of* Campylobacter-*positive samples (all producers): (A) feces and (B) cecal samples.*

DISCUSSION

The high proportion of *Campylobacter*-positive flocks in this study (Table 1) is similar to the situation in many

TABLE 4. Campylobacter *contamination of transport coops, process water, and processed carcasses for each producer*

Sample type	Producer A	B	C	D
Pretransport coops	30.0[a]	6.2	11.3	28.6
Posttransport coops	85.0	58.8	42.5	85.0
Prescalding water	8.6	0	0	2.5
Postscalding water	25.0	0	0	7.5
Prechilling water	0	2.5	7.5	0
Postchilling water	0	7.5	2.5	15.0
Carcass rinse	30.0	24.6	21.0	40.9

[a] Percentage of positive samples.

other countries, although a lower prevalence has been reported in parts of Scandinavia *(1, 3).* Even in Scandinavia, however, colonization of broiler chickens is generally first evident at 3 to 4 weeks of age, as has been observed elsewhere *(2, 7, 9, 13).* Poultry are rarely found to be positive during the first 2 weeks of life, but the point at which those destined to become positive begin to shed the organisms is considered important, because it may be possible to delay the event sufficiently to avoid any contaminated or carrier birds entering the processing plant. Several studies have shown that colonization of flocks can be postponed by vigorous cleaning and disinfection of poultry houses, use of disinfectant foot baths, and other biosecurity measures *(7, 8, 17, 21).*

The study described here was carried out on poultry flocks for which husbandry conditions were known to be different in some respects from those of major European producers. For example, completely fresh litter was not always used for each flock, cleaning and disinfection of the houses were generally more limited, and ventilation had to be increased in hot weather by opening parts of the houses to the exterior. Despite those differences and the use of flocks at high and low growth performance sites, the rate of onset of *Campylobacter* shedding was similar to, or even lower than, that observed in the European studies. It is not known whether any changes in current U.S. husbandry practices would delay shedding further, and the possibility needs to be investigated.

There is also uncertainty about the exact source(s) of flock infection. The rearing environment was sampled extensively, both inside and outside the houses, but in only three flocks were positive samples obtained from within the house before the birds became shedders. Since the infective dose for chicks can be as low as 35 cells *(19),* it is possible that present sampling and isolation methods are inadequate to detect very low levels of contamination, particularly for cells that could be sublethally injured from environmental exposure and therefore less likely to be recovered with the use of selective isolation media. It may be significant that, in two of the three flocks, the positive environmental samples were drag swabs (Table 2), which covered a large surface area. The relationship between environmental contamination and subsequent flock infection is being investigated with the use of molecular subtyping of isolates and will be reported separately. It is evident, however, that environmental contamination is not always associated with flock infection (Table 3). Payne et al. *(14)* used litter from a *Campylobacter*-positive flock for experimental trials on chicks and found no transfer of infection over a 7-week period, whether the litter was taken immediately after removal of the original flock or up to 9 days later.

The apparent seasonal effect on *Campylobacter* spp. carriage in poultry and the difference between fecal and cecal samples in this respect need further investigation over a longer period. In the United Kingdom, Humphrey et al. *(7)* found no such seasonal effect, whereas Wallace et al. *(22),* in the same country, reported seasonal differences in carriage, with a summer peak, and varying levels in different parts of the alimentary tract. However, the apparent lower recovery rate of *Campylobacter* from the cecal droppings may be confounded by the total number sampled versus five times the number of fecal samples cultured. Also, the samples of fecal material were much larger than those of cecal droppings.

Results obtained for the transportation of birds to the processing plant (Table 4) showed that the coops were not being effectively cleaned beforehand and could be a vehicle for cross-contamination, particularly for flocks that were *Campylobacter* negative at harvesting. The sporadic recovery rate before transport versus posttransport may be influenced by the condition of the sample, in which more injured cells may make recovery difficult. Since the entire cage was not swabbed, the site of sampling may also influence recovery. Nevertheless, only one of the four negative flocks acquired *Campylobacter* after leaving the farm. Following the processing of all flocks, the overall proportion of positive carcasses was relatively low (21.0 to 40.9%) in relation to the high incidence of positive flocks and may have been influenced by the delay in examining samples after collection or the handling conditions in the processing plant. For a number of flocks, there was an additional 24- to 48-h delay in transport of the samples, which was beyond control of the authors. These delays resulted in prolonged exposure of samples to ambient temperature and probably led to reduced recoveries of *Campylobacter.* Also, it is possible that prolonged contact between carcasses and chlorinated water in the immersion chilling system was another factor, especially in view of recent increases (from as little as 0 ppm to a consistent 50 ppm) in chlorine concentrations used by some processors. Further reductions in carcass contamination are likely to require specific control measures, both on the farm and in the processing plant, but more work is needed to clarify the exact requirements in each case.

ACKNOWLEDGMENTS

The authors are most indebted to the participating companies for their help in this study and willing cooperation. The technical support of Susan Brooks, Carolyn Gilbert, Donna Hinson, Leena Jain, Jennifer Murphy, Debbie Posey, Lalla Tanner, Matthew Tanner, and Calvin Williams is gratefully acknowledged. In addition to our ARS-appropriated funding, portions of the resources for this project were provided through FSIS contract no. 53-3A94-97-10.

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